ISSN: 1984-5529

Científica

Jaboticabal

v.43, n.2, p.156–164, 2015

http://dx.doi.org/10.15361/1984-5529.2015v43n2p156-164

Toxicity of biofertilizers on seeds of lettuce and maize

Toxicidade de biofertilizantes sobre sementes de alface e milho

Ariane SPIASSI[1]; Francielly Torres dos SANTOS[2]; Lúcia Helena Pereira NÓBREGA[3]; Taiana CESTONARO[4]; Mônica Sarolli Silva de Mendonça COSTA[5]

[1] Bióloga; Doutoranda pelo programa de Pós-graduação em Engenharia Agrícola (PGEAGRI); Universidade Estadual do Oeste do Paraná (UNIOESTE); Rua Universitária, n° 2069, Bairro Jardim Universitário, Cascavel/PR. CEP 85819-110; e-mail: arispiassi@hotmail.com
[2] Tecnóloga em Alimentos; Doutoranda pelo PGEAGRI; UNIOESTE; francielly_torres@hotmail.com
[3] Engenheira Agrônoma; Doutora; Profa. Associada do PGEAGRI; UNIOESTE; lucia.nobrega@unioeste.br
[4] Engenheira Ambiental; Doutoranda em Saneamento, Meio ambiente e Recursos Hídricos; Universidade Federal de Minas Gerais (UFMG); tcestonaro@hotmail.com
[5] Engenheira Agrícola; Doutora; Profa. Adjunta do PGEAGRI; UNIOESTE; msscm@ig.com.br

Recebido em: 31-03-2014; Aceito em: 08-12-2014

Abstract

This study evaluated the toxicity of biofertilizers using lettuce seed as bioindicator and maize as cropped plant. The experiment was carried out with five treatments consisting of biofertilizers and manure obtained from mixtures of cattle and ovine litter: T1 - 100% cattle manure; T2 - 50% cattle manure and 50% ovine litter; T3 - 75% cattle manure and 25% ovine litter; T4 - 75% ovine litter and cattle 25%; T5 - 100% ovine litter and T0 control - distilled water. Five concentrations of each treatment were used with 3, 6, 9, 12 and 15% in three replications submitted to biological testing. The use of biofertilizers with high EC negatively influences lettuce seed germination, the shoots of seedlings when diluted act as a stimulant. The biofertilizer did not affect the maize seeds germination and the seedlings growth was stimulated.

Additional keywords: cattle manure; conductivity; ovine litter.

Resumo

O objetivo deste trabalho foi verificar a toxicidade de biofertilizantes utilizando sementes de alface como bioindicadoras e de milho como planta cultivada. O experimento contou com cinco tratamentos constituídos por biofertilizantes obtidos de misturas de dejetos de bovinos e cama de ovinos: T_1 - 100% dejeto bovino; T_2 - 50% dejeto bovino e 50% cama de ovino; T_3 - 75% dejeto bovino e 25% cama de ovino; T_4 - 75% cama de ovino e 25% dejeto bovino; T_5 - 100% cama de ovino e a testemunha T_0 - água destilada. Cinco concentrações de cada tratamento foram utilizadas sendo elas 3, 6, 9, 12 e 15%, com três repetições, submetidas ao teste biológico. Os resultados mostram que a utilização de biofertilizantes com alta C.E. influencia negativamente a porcentagem de germinação das sementes de alface, mas agem como estimulantes do comprimento de parte aérea de plântulas. Em sementes de milho, o uso dos biofertilizantes não influencia a porcentagem de germinação, porém o crescimento de plântulas é estimulado.

Palavras-chave adicionais: cama de ovino; condutividade elétrica; dejeto bovino.

Introduction

Brazil has undergone technological changes in the recent years, aiming to increase productivity and reduce production costs in agriculture. As the high consumption of animal protein and the availability of area for production did not follow the activity growth, many producers were forced to change the rearing system, not only as a way to improve productivity, but also to increase the economic return speed of the activity (ASSOCON, 2012). Thus, the intensive rearing of cattle for slaughter has contributed to the emergence of serious environmental problems due to the high waste disposal and lack of paving of the confinements (Ribeiro et al., 2007).

Ovine raising has greater representation in the states of Bahia, Ceará, Piauí, Pernambuco, Rio Grande do Norte, Rio Grande do Sul, Paraná and Mato Grosso do Sul. The annual production reaches 11 million tons of wool, mainly in Rio Grande do Sul, with production chain formed by 35 thousand agricultural establishments (Brasil, 2013). For the sector to keep a satisfactory performance, it is necessary to invest in productivity and cost reduction. In addition, it is necessary to pay special attention to environmental issues, emphasizing the importance of the use of waste from agricultural industries (Nunes et al., 2005). An alternative in the use and treatment of agro-industrial waste is to submit them to the anaerobic biodigestion process.

According to Côte et al. (2006) the anaerobic biodigestion may be defined as a complex interaction of microorganisms that degrade the various organic compounds present in the waste to the final form of methane and carbon dioxide, mainly. Different species of microorganisms act in this process, being the non-methanogens responsible for hydrolyzing organic polymers and fermenting them into acetate, hydrogen and carbon dioxide. As for the methanogens, they convert these byproducts into methane.

The nutrients contained in the waste ensure the survival and reproduction of microorganisms present during anaerobic biodigestion, encouraging the degradation of the non-stable organic fraction, and, therefore, pollutant, to the stable form, the biofertilizer, besides producing biogas (Alvarez & Lidén, 2008). The bovine biofertilizers, being a source of bioactive compounds with bacteria, yeasts, algae and fungi, have positive action on plant nutrition and plant health and stimulate the release of humic substances into the soil (Mahmoud & Mohamed, 2008).

According to Maia et al. (2008) the use of effluents in agriculture has grown considerably in many countries, including Brazil. However, all the positive and negative aspects of this technique, especially on nutrient uptake by plants or their toxicity, have not been sufficiently studied yet.

To investigate the toxicity of a biofertilizer, it is necessary meet certain requirements. One of the tests included in the Canadian C. Environment Ministerial (CCME) (1996) recommends that "the germination of indicator seeds in aqueous extracts of organic compounds should reach values above 90% germination compared to control". The germination index is an organic chemical condition that indicates the presence or absence of organic compost that can cause toxicity (Silva & Villas Bôas, 2007).

In this context, this study aimed to evaluate the toxicity of biofertilizers from the anaerobic biodigestion of bovine manure and sheep litter using lettuce (*Lactuca sativa* L.) as a bioindicator and maize (*Zea mays* L.), as cropped plant.

Material and methods

The experiment was carried out in the Laboratories of Agro-industrial Waste Analysis (LARA) and Seed and Plant Assessment (LASP), both belonging to Center of Exact and Technological Sciences, of Universidade Estadual do Oeste do Paraná - UNIOESTE, Cascavel city, Brasil, located at Latitude 24º53'S, Longitude 53º23'W and Altitude of 682 meters.

The biofertilizers used in the tests were obtained through the anaerobic biodigestion process. The ratio used for the formulation of the biodigester effluents containing cattle manure and ovine litter sought the percentage of total solids between 5 and 8%. The hydraulic retention time was 159 days in batch system (Cestonaro, 2013). The treatments included proportions of cattle manure and ovine litter, as follows: T_0 - distilled water; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% cattle manure and 25% ovine litter; T_4 - 75% ovine litter and 25% cattle manure and T_5 - 100% ovine litter.

The characterization of biofertilizers from the biodigestion of cattle manure and ovine litter was made for total and volatile solids and biochemical oxygen demand (BOD), according to the methodology described by the APHA (2012). The determination of nitrogen, phosphorus, potassium, sodium, calcium, magnesium, manganese, iron, zinc and copper were according to methodology of Malavolta (1989). Cellulose and hemicellulose were obtained through the determination of neutral detergent fiber (NDF) and acid detergent fiber (ADF), according to Campos et al. (2004). Fractionation of organic matter, as well as the carbon quantification of the fractions of fulvic acids (FA) and humic acid (HA), to obtain the ratio HA/FA, was performed according to the methodology proposed by Benites et al. (2003) (Table 1).

Table 1 - Characteristics of biofertilizers obtained through the anaerobic biodigestion of cattle manure and ovine litter.

Treatments	Parameters								
	pH	E.C.[c] (dS m^{-1})	TKN[a] (%)	P[b]	K[b]	COD[b]	HA/FA	Cel.[a] (%)	Hemi.[a] (%)
				--------- (g L^{-1}) ---------					
T_1	7.61	11.23	0.18	0.55	2.71	8.21	3.02	10.30	12.32
T_2	7.65	11.13	0.16	0.56	2.32	7.71	2.55	12.39	13.18
T_3	7.53	12.03	0.14	0.48	1.83	6.62	2.34	13.99	14.04
T_4	7.39	11.68	0.11	0.39	1.38	6.33	1.47	16.06	15.75
T_5	7.26	12.01	0.09	0.34	0.97	5.16	1.21	17.64	16.05

E.C. - Electrical conductivity; TKN - Total Kjeldahl nitrogen; P - phosphorus; K - potassium; COD - chemical oxygen demand; HA/FA - humic acids/fulvic acids; Cel - Cellulose; Hemi - Hemicellulose; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% ovine manure and 25% ovinelitter; T_4 - 75% ovine litter and 25% cattle manure; T_5 - 100% ovine litter.

The concentrations used for each treatment were 3; 6; 9; 12 and 15%. Table 2 shows the electrical conductivity (E.C.) of each treatment. After dilution, the E.C. of the solution was measured by a conductivity meter reading, Digimed mark, CD-21 model, being the results expressed in $dS.m^{-1}.g^{-1}$.

Table 2 - Electrical conductivity of treatments in each dilution.

Treat./ Conc.	E.C. ($dS\ m^{-1}$)	Treat./ Conc.	E.C. ($dS\ m^{-1}$)	Treat./ Conc.	E.C. ($dS\ m^{-1}$)	Treat./ Conc.	E.C. ($dS\ m^{-1}$)	Treat./ Conc.	E.C. ($dS\ m^{-1}$)
T_1 - 3%	0.33	T_2 - 3%	0.37	T_3 - 3%	0.34	T_4 - 3%	0.32	T_5 - 3%	0.49
T_1 - 6%	0.64	T_2 - 6%	0.71	T_3 - 6%	0.67	T_4 - 6%	0.75	T_5 - 6%	0.78
T_1 - 9%	0.94	T_2 - 9%	1.02	T_3 - 9%	0.98	T_4 - 9%	1.08	T_5 - 9%	1.15
T_1 - 12%	1.21	T_2 - 12%	1.36	T_3 - 12%	1.26	T_4 - 12%	1.37	T_5 - 12%	1.56
T_1 - 15%	1.50	T_2 - 15%	1.68	T_3 - 15%	1.53	T_4 - 15%	1.66	T_5 - 15%	1.79

Treat./Conc. – Treatment – Concentration; E.C. - Electrical conductivity; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% cattle manure and 25% ovine litter; T_4 - 75% ovine litter and 25% cattle manure; T_5 - 100% ovine litter.

The lettuce and maize seeds used in the experiment were purchased in local trade. For lettuce, glass Petri dishes, with a diameter of 100 mm and height of 15 mm, lined with two sterile filter paper sheets, were used. 3.0 mL of the solution obtained in the previous step were added, making sure there was no formation of air bubbles in order to ensure seed contact with biofertilizers.

The solution was added only once, at the beginning of bioassays, and, from then on, only distilled water was added whenever it was necessary (Souza Filho et al., 2010). Immediately after adding the substrate on the Petri dish, using tweezers, 20 seeds were distributed on the paper, spacing them to ensure growth. The plates were placed in a vertical incubator (BOD). The seeds remained at a temperature of 25 ^{0}C under a photoperiod of 12 h, counting was performed daily until the 7th day of germination (adapted from Brasil, 2009).

Maize seeds without chemical treatment, from the hybrid CD 384 Hx harvest 2012/2012, were used in order to verify if the biofertilizers interfere with germination and early seedling development. Moistened paper roll for germination was used as sowing substrate in the ratio of two and a half times the volume of distilled water (control) or treatments for the mass of the paper (Marcos Filho et al., 1987). The rollers were made of four sheets of paper, having two as the base for the distribution of seed and two cover sheets, and then were placed in BOD. Evaluations of seeds and seedlings were made according to the criteria of the Rules for Seed Analysis (Brasil, 2009).

The counting of germinated seeds was obtained from the first day after the emergence of the radicle until the stabilization of the number of seedlings, following the methodology described in Nakagawa (1999). Seeds presenting radicle length of 2 mm were considered to be germinated. The germination speed index (GSI) was calculated according to Maguire (1962).

Data used to evaluate the germination speed were the same as for the evaluation of GSI. The germination speed (GS) was calculated according to Edmond & Drapala (1958).

After the growth period, as described above, it was determined the primary root length (cm) and hypocotyl length (cm) of ten normal seedlings of each repetition. Measurements were made using a graduated ruler (accuracy of 1.0 mm). Then it was determined the fresh mass of root and aerial part of maize and fresh mass of the whole seedling for lettuce because of its small size. For dry mass, the seedlings were placed in paper bags properly identified and taken to the oven at 60 °C for 48 h. The weighing was carried out on a 0.001 g precision scale, determining the total dry mass of ten seedlings for each repetition.

The experimental design was completely randomized (DCR), with five treatments and five dilutions, with three repetitions and two species (one bioindicator and one cropped). A control containing only distilled water was used. Data were submitted to descriptive statistics and normality analysis through Minitab 14 program. Data showing abnormalities (p-value less than 0.05) were transformed by $\sqrt{x+0,5}$. The data expressed as percentage were transformed by arc sin $\sqrt{x/100}$ (Banzatto & Kronka, 1995; Brasil, 2009).

The analysis of variance (ANOVA) and data transformation presented as abnormal were performed using the statistical program SISVAR (Ferreira, 2008). The comparison of treatment means was performed with the application of Skott-Knott test at 5% probability.

Results and discussions

Table 3 shows the results for germination percentage; germination speed index and germination speed using biofertilizers on lettuce seeds. Regardless the concentration of biofertilizers, there was lettuce seed germination in all treatments.

Table 3 - Average values for germination percentage (%G), germination speed index (GSI) and germination speed (GS) using lettuce seeds as a bioindicator to evaluate the toxicity of biofertilizers.

Treatment	Concentration (%)	Germination (%)	GSI [n.s.]	GS [n.s.]
Control	0	71.7 a	4.49	3.44
T_1	3	73.3 a	4.62	3.56
	6	53.3 b	3.39	3.48
	9	56.7 a	3.67	3.56
	12	35.0 b	2.21	3.39
	15	38.3 b	2.61	3.21
T_2	3	55.0 a	3.55	3.59
	6	53.3 b	3.16	3.66
	9	66.7 a	4.26	3.58
	12	53.3 b	3.46	3.39
	15	63.3 a	3.46	3.39
T_3	3	50.0 b	2.89	3.60
	6	43.3 b	2.76	3.34
	9	46.7 b	3.40	3.00
	12	40.0 b	3.17	2.70
	15	38.3 b	2.44	3.53
T_4	3	56.7 a	3.41	3.65
	6	65.0 a	3.85	3.72
	9	46.7 b	3.14	3.29
	12	60.0 a	4.01	3.42
	15	56.7 a	3.58	3.49
T_5	3	65.0 a	4.19	3.43
	6	51.7 b	3.22	3.47
	9	48.3 b	3.54	3.04
	12	70.0 a	4.80	3.27
	15	58.3 a	3.68	3.65
General CV (%)		15.40	23.24	9.91
General Average		54.48	3.50	3.42

Note: different letters correspond to different means at 5% significance level by Scott-Knott test; *n.s. - non significant at 5% probability. Control - distilled water; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% sheep litter; T_3 - 75% cattle manure and 25% sheep litter; T_4 - 75% sheep litter and 25% cattle manure; T_5 - 100% sheep litter.

Note that in T1 the germination percentage was significantly different from the control, and the lowest percentage was observed in the concentration 12%, followed by concentration 15% and 6%. It is observed that the higher the E.C., the lower the germination percentage, which can be confirmed in T1 and T3. Considering that the high E.C. may reflect the toxicity of biofertilizers, since the toxicity is a property that demonstrates the potential of a substance to cause harmful effect to a living organism. E.C. is the concentration of chemicals, such as salts, that interfere with water absorption by the roots, causing, thus, seedling dehydration (Rand, 1995; Costa et al., 2008).

It is observed in T3 that the germination percentage compared to the control has lower average values. This indicates that this treatment has a higher potential of toxicity to lettuce seeds.

T4 showed higher germination percentage, except for the concentration 9%, when compared to the others. Thus, T4 composition does not show toxic potential for the seed germination.

However, in T5 higher germination percentages were recorded for the highest concentrations of biofertilizers. As observed on Table 1, T5 has in its composition low values for N-P-K. The highest germination percentage, in this case, is related to the initial phase of the seeds, in which they do not require high amounts of nutrients, because they have their natural reserves. However, Souza et al. (2005) ensure that seeds are excellent organisms for bioassays, because when they are rehydrated they start a germination process in which they undergo rapid physiological changes and become highly sensitive to environmental stress. Thus, attesting the importance of using seeds, once they are sensitive to verify biofertilizer toxicity.

In contrast to the results found in this study, Paixão Filho et al. (2008), using the germination test to check the toxicity of effluents of anaerobic filter, concluded that the effluent did not show any toxicity degree, since the increased concentration of the effluent did not reduce the germination of lettuce seeds used as bioindicators.

Young et al. (2012) reported that the use of a simple tool like the toxicity test with lettuce seeds allows the assessment of the quantity and efficiency of the waste treatment system. In this case, anaerobic digestion, suggesting that after the determination of toxicity reduction, by dilution, the effluent can be used in soil irrigation.

For the germination speed index and germination speed (Table 3), there was no statistically significant difference between all treatments and their concentrations, with control containing only distilled water, in the hydration of the tested seeds.

The treatments (different proportions of cattle manure and ovine litter treated by anaerobic digestion) and tested concentrations (3, 6, 9, 12 and 15%), did not affect significantly the evaluated characteristics of root length, fresh mass and dry mass of lettuce seedlings. However, there was a significant effect for the lettuce seedlings in the length of aerial part, shown on Table 4.

For treatments 2 and 5, it is observed that the higher the concentration (9, 12 and 15%) the higher the length of aerial part, compared to the control. Viana et al. (2001) studied the influence of water salinity, with the E.C. ranging from 0.3 to 3.8 dS.m^{-1} on the lettuce seeds, and found that the high E.C. negatively affected the length of the root and aerial part of the seedlings.

Comparing the effects of E.C. on the variables considered in the current studies, it is verified that there have been more intense effect on the germination indices (GSI and GS) than on the seedling growth (length of root and aerial part), considering the sensitivity of lettuce seeds in the initial phase regarding the effluent salinity.

Table 4 - Average values for root and aerial part length, fresh mass and dry mass of lettuce seedlings used as bioindicators to assess the biofertilizers toxicity.

Treatment	Concentration (%)	Length (cm)		Mass (g)	
		Root $^{n.s.}$	Aerial part	Fresh mass $^{n.s.}$	Dry mass $^{n.s.}$
Control	0	0.52	0.49 b	0.1567	0.0022
T_1	3	0.74	0.67 b	0.0392	0.0024
	6	0.65	1.03 a	0.0727	0.0046
	9	0.61	0.97 a	0.0673	0.0050
	12	0.34	0.46 b	0.0279	0.0020
	15	0.24	0.57 b	0.0371	0.0028
T_2	3	0.52	0.46 b	0.0397	0.0028
	6	2.98	0.67 b	0.0426	0.0035
	9	0.84	1.15 a	0.2533	0.0183
	12	0.75	0.84 a	0.0460	0.0041
	15	0.69	1.14 a	0.0618	0.0045
T_3	3	0.28	0.29 b	0.0236	0.0016
	6	0.41	0.50 b	0.0341	0.0019
	9	0.61	0.72 b	0.0472	0.0025
	12	0.63	0.97 a	0.0760	0.0041
	15	0.47	0.46 b	0.0297	0.0021
T_4	3	0.74	0.77 b	0.0337	0.0034
	6	0.69	0.71 b	0.0353	0.0023
	9	1.03	1.34 a	0.0421	0.0036
	12	1.35	1.11 a	0.0783	0.0049
	15	0.62	0.83 b	0.2370	0.0043
T_5	3	1.98	1.12 a	0.0780	0.0054
	6	0.51	0.59 b	0.0438	0.0035
	9	0.96	0.95 a	0.0642	0.0043
	12	0.95	1.22 a	0.0905	0.0047
	15	0.79	1.23 a	0.0690	0.0055
General CV (%)		3.97	40.14	51.08	43.83
General Average		0.83	0.82	0.2361	0.058

Note: different letters correspond to different means at 5% probability by Scott-Knott test; *n.s. - non significant at 5% probability; Control - distilled water; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% cattle manure and 25% ovine litter; T_4 - 75% ovine litter and 25% cattle manure; T_5 - 100% ovine litter.

The highest E.C. found in this study was 1.79 dS m^{-1}, for T5 in the concentration of 15% (Table 2), this E.C. value provided growth of root and aerial part of lettuce seedlings in this concentration of biofertilizers. Since Costa et al. (2001) stated that the E.C. variation of the nutritive solution changes the water and nutrients by the plants, interfering with their metabolism and, consequently, their production, and may then be detrimental to values around 2.5 dS m^{-1}.

Viana et al. (2001) analyzed the influence of saline water, in irrigation with E.C. ranging from 0.3 to 3.8 dS m^{-1} on lettuce seeds. The authors founded that the high E.C. negatively affected the root and aerial part length of the seedlings.

In addition to using lettuce seeds as bioindicators, it is important to perform the toxicity test in a crop, like maize used in this study, since it is one of the most cropped in the study region.

Table 5 shows the germination percentage, the germination speed index, the germination speed and the root and aerial part length of maize under treatments with biofertilizers concentrations.

Table 5 - Average values for germination percentage (%G), germination speed index (GSI), germination speed (GS) and root and aerial part length of maize seedlings, using biofertilizers concentrations (Conc.).

Treatment	Conc. (%)	G (%) $^{n.s}$	GSI $^{n.s.}$	GS $^{n.s.}$	Length (cm)	
					Root	Aerial part
Control	0	100	24.17	2.10	14.27 b	7.01 b
T_1	3	100	24.28	2.08	13.85 b	10.17 a
	6	100	24.50	2.06	15.67 a	9.84 a
	9	100	24.22	2.09	14.59 b	10.42 a
	12	100	23.72	2.15	14.18 b	9.91 a
	15	100	23.94	2.14	16.43 a	9.83 a
T_2	3	100	24.17	2.10	14.18 b	8.07 b
	6	100	24.39	2.07	16.17 a	8.75 b
	9	100	24.11	2.10	15.83 a	7.84 b
	12	100	24.33	2.08	15.65 a	8.32 b
	15	100	23.78	2.14	16.32 a	8.67 b
T_3	3	100	24.11	2.10	15.64 a	10.22 a
	6	100	23.88	2.13	13.03 b	10.07 a
	9	100	24.28	2.09	13.16 b	9.87 a
	12	100	24.50	2.06	14.39 b	10.42 a
	15	100	24.50	2.06	13.24 b	9.80 a
T_4	3	100	23.78	2.14	19.36 a	10.57 a
	6	100	24.22	2.09	17.48 a	10.62 a
	9	100	24.11	2.10	17.62 a	9.64 a
	12	100	23.89	2.13	15.41 a	10.20 a
	15	100	24.00	2.12	18.42 a	9.90 a
T_5	3	100	24.16	2.10	14.02 b	9.57 a
	6	100	24.11	2.10	15.49 a	9.97 a
	9	100	24.17	2.10	15.15 a	10.30 a
	12	100	24.17	2.10	13.22 b	10.15 a
	15	100	24.00	2.12	12.65 b	12.71 a
General CV (%)		0	1.74	2.39	6.00	7.07
General Average		100	24.14	2.10	15.27	9.73

Note: different letters correspond to different means at 5% probability by Scott-Knott test; *n.s. - non significant at 5% probability; Control - distilled water; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% cattle manure and 25% ovine litter; T_4 - 75% ovine litter and 25% cattle manure; T_5 - 100% ovine litter.

For the germination percentage of maize seeds, the concentrations and treatments did not show toxic potential that interfere with seed germination. This fact is because the seed is less sensitive to high E.C. T4 (Table 5) showed the highest means for length of root and aerial part, compared to the control, without presenting statistical difference between their concentrations, differing from the control.

T3 (Table 5) was the treatment that presented the highest number of concentrations with average root length statistically equivalent to the control. For the length of aerial part, it showed higher values than the control. Therefore, the E.C. positively affects the growth of the roots. Only in T2, this statement is reversed, with lower averages in aerial part (8.33 cm) and increase in root length (15.63 cm).

Regarding the development of maize seedlings, the results are shown on Table 6.

Table 6 - Average values for fresh mass and dry mass of roots and aerial part of maize seedlings, using biofertilizers concentrations.

Treatment	Conc. (%)	Fresh mass (g)		Dry mass (g)	
		Root	Aerial part	Root	Aerial part
Control	0	4.42 b	2.83 b	0.46 a	0.23 g
T_1	3	5.05 a	3.85 b	0.34 e	0.32 c
	6	4.90 b	3.69 b	0.47 a	0.30 d
	9	4.78 b	4.43 a	0.42 b	0.37 a
	12	4.66 b	4.15 a	0.47 a	0.39 a
	15	4.68 b	3.78 b	0.36 d	0.27 f
T_2	3	5.45 a	3.57 b	0.46 a	0.21 h
	6	5.03 a	3.70 b	0.39 c	0.25 f
	9	5.30 a	3.64 b	0.33 e	0.23 g
	12	5.36 a	3.73 b	0.30 f	0.22 g
	15	4.72 b	4.05 b	0.43 c	0.28 f
T_3	3	4.33 b	3.81 b	0.35 d	0.29 e
	6	4.85 b	4.11 a	0.41 c	0.31 d
	9	4.69 b	4.54 a	0.45 a	0.38 a
	12	4.58 b	5.07 a	0.43 c	0.37 a
	15	4.42 b	4.83 a	0.41 c	0.33 c
T_4	3	5.10 a	3.93 b	0.37 d	0.33 c
	6	4.99 a	4.59 a	0.36 d	0.31 d
	9	4.45 b	4.74 a	0.41 c	0.28 e
	12	4.72 b	4.63 a	0.39 c	0.34 b
	15	4.75 b	4.19 a	0.41 c	0.29 d
T_5	3	5.36 a	4.36 a	0.48 a	0.33 c
	6	5.32 a	4.48 a	0.48 a	0.35 b
	9	4.52 b	4.27 a	0.46 a	0.33 c
	12	4.52 b	4.18 a	0.42 c	0.31 d
	15	4.68 b	4.03 b	0.43 c	0.34 b
General CV (%)		8.06	11.00	4.77	3.21
General Average		4.83	4.12	0.41	0.30

Note: different letters correspond to different means at 5% probability by Scott-Knott test; Control - distilled water; T_1 - 100% cattle manure; T_2 - 50% cattle manure and 50% ovine litter; T_3 - 75% cattle manure and 25% ovine litter; T_4 - 75% ovine litter and 25% cattle manure; T_5 - 100% ovine litter.

The fresh mass of root and aerial part of maize seedlings was not negatively affected by the treatments, being equal or higher when compared to the control. In general, it is observed that the fresh mass of root becomes smaller as the E.C. increases. This fact is reported in all treatments, since the amount of salts available to the root may affect its development.

For fresh mass of aerial part, treatments 3, 4 and 5 showed the highest means when compared with the control, not differing in higher concentrations, increasing the seedling weight even under high E.C.

The greatest statistical differences were observed in dry mass of root of maize seedlings. T2, T3 and T4 showed lower means when statistically compared to the control. The dry mass of aerial part increased with the use of the treatments compared to the control. Whereas T1 and T3, in concentrations 9 to 12%, had higher dry mass values of aerial part.

Although the dry mass of root has been affected by the E.C., this fact is not limiting for the use of biofertilizers, since the culture is less sensitive to the E.C.

Conclusion

The use of biofertilizers with high E.C. negatively influences the germination percentage of lettuce seeds, but acts as stimulant of the length of aerial part of seedlings. In maize seeds, the use of biofertilizers does not influence the germination percentage, but seedling growth is stimulated. Thus, its use as a source of nutrients to the crop can be recommended.

Acknowledgment

To Agricultural Engineering Graduate Program (PGEAGRI/UNIOESTE) for opportunity. To CAPES for granting Masters Scholarship.

References

APHA (2012) American Public Health Association, AWWA, WEF, Standard methods for the examination of water and wastewater. APHA, Washington. 1496p.

ASSOCON (2012) Associação Nacional dos Confinadores. Disponível em <http://www.assocon.com.br/not%2046%202.htm>. (Acesso em 11 nov. 2013).

Alvarez R, Lidén G (2008) Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renewable Energy 33(2):726-734.

Banzatto DA, Kronka SN (1995) Experimentação agrícola. Funep, Jaboticabal. 247p.

Benites VM, Madari B, Machado PLA. (2003) Extração e fracionamento quantitativo de substâncias húmicas do solo: um procedimento simplificado de baixo custo. Rio de Janeiro: Embrapa Solos. 7 p. (Comunicado Técnico 16).

Brasil (2009) Ministério da Agricultura, Pecuária e Abastecimento. Regras para análise de sementes. Secretaria de Defesa Agropecuária. Mapa/ACS. 365p.

Brasil (2013) Ministério da Agricultura Pecuária e Abastecimento – MAPA. Caprinos e ovinos. Disponível em <http://www.agricultura.gov.br/animal/especies/caprinos-e-ovinos>. (Acesso em 04 nov. 2013).

CCME (1996) Canadian C. Enviroment Ministerial. Guidelines for compost quality. Toronto. 106p.

Campos FP, Nussio CMB, Nussio LG (2004) Métodos de análise de alimentos. FEALQ, Piracicaba. 135p.

Costa PC, Didone EB; Sesso TM (2001) Condutividade elétrica da solução nutritiva e produção de alface em hidroponia. Ciência Agrícola 58(3):595-597.

Costa CR, Olivi P, Botta CMR, Espindola ELG (2008) A toxicidade em ambientes aquáticos: discussão e métodos de avaliação. Revista Química Nova 31(7):1820-1830.

Cestonaro T (2013) Adição de dejetos de bovinos de corte em processos de estabilização de cama de ovinos. Unioeste (Dissertação de Mestrado Engenharia Agrícola).

Côte C, Masse DI, Quessy S (2006) Reduction of indicator and pathogenic microorganisms bypsychrophilic anaerobic digestion in swine slurries. Bioresource Technology 97(1):686-691.

Edmond JB, Drapala WJ (1958) The effects of temperature, sand and soil, and acetone on germination of okra seed. Proceedings of the American Society Horticutural s.v.(71):428-434.

Ferreira DF (2008) Manual do sistema Sisvar para análises estatísticas. UFLA, Lavras. 66p.

Maguire JD (1962) Seeds of germination-aid selection and evaluation seedling emergence and vigor. Crop Science 2(s.n.):176-177.

Mahmoud AA, Mohamed HF (2008) Impact of biofertilizers application on improving wheat (*Triticum aestivum* L.) resistance to salinity. Research Journal of Agriculture and Biological Sciences 4(5):520-528.

Maia SSS, Azevedo CMSB, Silva FN (2008) Efeito do efluente de viveiro de peixe na composição de biofertilizantes na cultura da alface. Revista Verde de Agroecologia e Desenvolvimento Sustentável. Grupo Verde de Agricultura Alternativa (GVAA) s.v.(2):36-43.

Malavolta E (1989) ABC da adubação. Agronomica Ceres, São Paulo. 201p.

Marcos Filho J, Cícero SM, Silva WR (1987) Avaliação da qualidade das sementes. FEALQ, Piracicaba. 230p.

Nakagawa J (1999) Testes de vigor baseados no desempenho das plântulas (1999) cap 2. In: Krzyzanowski FC, Vieira RD, Neto JBF Vigor de sementes: conceitos e testes. Associação Brasileira de Tecnologia de Sementes, Comitê de vigor de sementes. ABRATES, Londrina 246p.

Nunes RV, Pozza PC, Nunes CGV (2005) Energy values of animal by-products for poultry. Revista Brasileira de Zootecnia 34(4):1217-1224.

Paixão Filho JL, Pires MSG, Gabrielli G (2008) Avaliação da toxicidade de efluentes sanitário de filtro anaeróbio com sementes de alface (*Lactuca Sativa)* para reuso agrícola. XVII Congresso interno de iniciação científica, Faculdade de Tecnologia, Campinas, UNICAMP.

Rand GM (1995) Fundamentals of aquatic toxicology: effects, environment fate, and risk assessment, 2 ed. London, Taylor & Francis, pp. 3-66.

Ribeiro GM, Sampaio AAM, Fernandes ARM, Henrique W, Sugohara, A, Amorim AC (2007) Efeito da fonte proteica e do processamento físico do concentrado sobre a terminação de bovinos jovens confinados e o impacto ambiental dos dejetos. Revista Brasileira de Zootecnia 36(6):2.082-2.091.

Silva FAM, Villas Bôas, RL (2007) Teste de germinação como indicador de manutenção em composto orgânico. Revista Energia na Agricultura 22(3):63-73.

Souza SAM, Stein VC, Cattelan LV (2005) Utilização de sementes de alface e de rúcula como ensaios biológicos para avaliação do efeito citotóxico e alopático aquoso de plantas medicinais. Revista de Biologia e Ciência da Terra 5(s.n.):01- 8.

Souza Filho APS, Guilhon GMSP, Santos LS (2010) Metodologias empregadas em estudos de avaliação da atividade alelopática em condições de laboratório: revisão crítica. Planta Daninha 28(3):689-697.

Viana SBA, Fernandes PD, Gheyi HR (2001) Germinação e formação de mudas de alface em diferentes níveis de salinidade de água. Revista Brasileira de Engenharia Agrícola e Ambiental, 5(2):259-264.

Young BJ, Riera NI, Beily ME (2012) Toxicity of the effluent from an anaerobic bioreactor treating cereal residues on *Lactuca sativa*. Ecoloxicology And Environmental Safety 76(s.n.):182-186.